Scanning Microscopy

Volume 3 | Number 3 Article 18

10-9-1989

The Physical State of Potassium in the Human Lymphocyte: A Review

William Negendank
Wayne State University

Scanning Microscopy, Vol. 3, No. 3, 1989 (Pages 865-875)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/89$3.00+.00

THE PHYSICAL STATE OF POTASSIUM IN THE HUMAN LYMPHOCYTE: A REVIEW

William Negendank

Division of Hematology-Oncology, Department of Medicine
Wayne State University
PO Box 02188
Detroit, Michigan 48201

Phone Number: 313-745-1395

(Received for publication May 4, 1989, and in revised form October 9, 1989)

Abstract

Studies of the effects of chemical potential, temperature, and metabolic perturbation on static ion contents, kinetics of the approach to equilibrium, and kinetics of ionic self-exchange in human lymphocytes are reviewed. The results contradict the classical concept of cell ion and water physiology, the membrane-osmotic, pump-leak theory, and are re-interpreted by an adsorption model of the cell. In this model, most of cell water exists in a physical state sufficiently ordered to reduce the partition function of dissolved ions, and most of cell potassium is associated with fixed charges on macromolecules. Competing adsorption of potassium and sodium is cooperative and has a critical temperature dependence. The kinetics of the approach to equilibrium are described by a time dependent Ising model. High rates of isotopic self-exchanges of potassium and sodium near the transition point are postulated to result from an increased rate of fluctuations within the ensemble of ion-adsorbing proteins.

Key Words: Potassium, Lymphocyte, Cooperative (Critical) transition, Ising Model

Introduction

The aqueous environment of most cells has a high concentration of sodium and a low concentration of potassium, while most cells have a low concentration of sodium and a high concentration of potassium. The classical explanation for this, as well as the relation between these ions and cell water content and volume, is the membrane-osmotic, pump-leak theory. This theory is based on the fundamental assumption that most of cell ions are freely dissolved within cell water, most of which exists in a physical state like that of water in a dilute solution. Fixed charges within the cell would impose a Donnan electrochemical equilibrium on the ionic distributions, but this would be incompatible with osmotic equilibrium and the cell would swell to destruction. An outwardly directed sodium pump within the surface membrane, subsequently identified as the Na,K-ATPase [43], overcomes this problem, and explains the maintenance of a low concentration of cell sodium in the face of its continual leakage into the cell [14, 45]. Potassium, chloride, and other ions tend to follow electrochemical equilibria, but in many cases modified by membrane pumps.

Three of the fundamental, experimentally-testable consequences of this theory are as follows:

1. Inhibition of the sodium pump permits cell swelling due to the forces involved in the Donnan equilibrium.
2. The gain of cell sodium caused by inhibition of the sodium pump is accompanied by a decreased rate of sodium efflux from the cell.
3. The rate-limiting steps in ion exchange are within the surface membrane, and manifested by single exponential functions in isotopic ion exchange experiments.

These concepts were tested in extensive studies of static ionic contents and rates of net and of isotopic ionic exchanges in human lymphocytes. The effects of chemical potential, temperature, perturbation by ouabain and valinomycin, and metabolic inhibition were determined. The results contradict all of these expectations based on the classical membrane-osmotic, pump-leak theory. The experiments are described in a number of papers [23, 24, 27-38] and earlier work, along with a detailed argument of its significance, was reviewed [22]. A briefer account was presented at the 4th Pfefferkorn Conference [25], so only a very brief outline will be mentioned here:

(1) Lymphocytes incubated at 0°C, incubated in

low external potassium, treated with ouabain, or depleted of ATP by metabolic inhibition gain sodium to concentrations higher than in the external medium. By this criterion they clearly have lost all ability to pump sodium out. However, under none of these circumstances did they gain water or swell [23]. The ad hoc postulation of volume-regulating mechanisms other than the Na,K-ATPase sodium pump, such as hydrostatic pressures, other pumps, altered charge on cell proteins, or shrunken and swollen compartments, could not account for the results.

(2) The gain of sodium under the abovementioned conditions was under no circumstances caused by a decrease in the efflux of sodium from the cell. In fact, efflux typically increased, while the cells were gaining sodium, as well as after a new high equilibrium level of sodium was achieved [31, 34, 35, 39]. The ad hoc postulation of mechanisms to account for this result, such as stimulation of a sodium pump by the high internal sodium concentration, a decreased internal negative charge, Na-K or Na-anion outward co-transport, Na-Ca or Na-H counter-transport, or an Na-Na exchange diffusion carrier, could not account for the results.

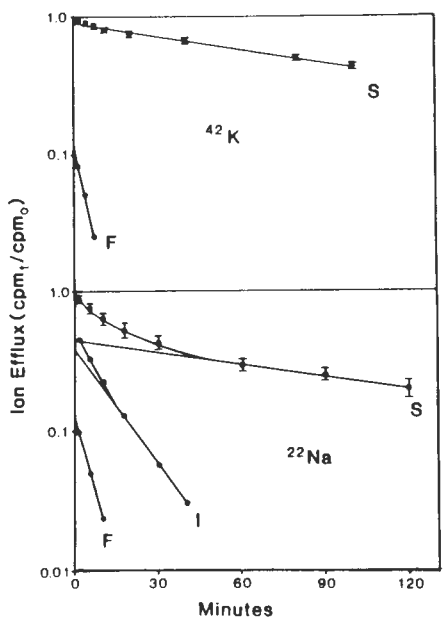


Figure 1. Self-exchange of potassium and sodium determined by isotopic efflux techniques in lymphocytes. Data are redrawn from reference 39.

(3) In kinetic ion exchange studies, in which cell ion concentrations are static but ionic self-exchange is determined by isotopic influx or efflux techniques, and in which cells are separated from the medium without washing and early time points are obtained, there appear fast fractions of ion exchange that are exponential and precede the dominant slower exponential fractions (Figure 1). The latter characterizes self-exchange of the majority of normal cell potassium [30] and about half of normal cell sodium [31] and chloride [24]. The fast fractions have been rigorously shown not to be experimental artifacts, contained in extracellular medium, attached to the outer surface of the membrane, or to result from subpopulations of small cells; but to be intracellular in origin and to exchange in series with the slower fractions. We have shown in extensive studies that the fast fractions correspond exactly to non-saturable fractions of ions within the cell [24, 30-35], and that they do in fact represent the permeability of the surface membrane. Since the slower fractions must therefore represent internal exchange mechanisms, and since the slower fractions are the ones that are most influenced by temperature, chemical potential, and metabolic perturbations, it is concluded that processes within the surface membrane do not actively control the cell levels of these ions.

These studies, and others reviewed elsewhere [22], have led us to reject the membrane-osmotic, pump-leak theory of cell ion exchange and volume control, as well as its fundamental assumption that the physical state of cell water and ions is primarily one of a dilute solution. The purpose of this paper is to review the physiological basis for a theory of the cell based on entirely different assumptions about the physical state of cell water and ions.

An Adsorption Model of Cell Potassium and Sodium

The adsorption model of the human lymphocyte, derived from earlier experimental data reviewed in reference 22 and additional data in references 24 and 39, is shown in Figure 2. Two kinds of observations form the primary basis for this model. First are the static ion concentrations studied as a function of the ratio of chemical potentials of potassium and sodium [29] (Figure 3). They show non-saturable fractions (S_{ns}) of both ions, and a saturable fraction (S_{sat}) of potassium that exchanges mole-for-mole with sodium at low concentrations of external potassium. The total amount of each cell ion is:

$$S_t = S_{ns} + S_{sat}. \quad (1)$$

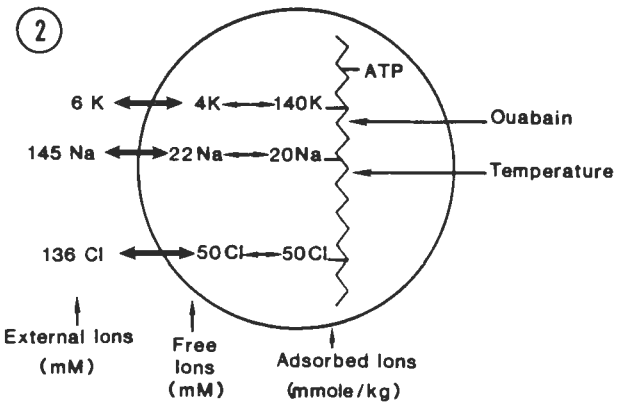
Ions in the nonsaturable fractions expressed as mmoles / liter cell water have ratios to the concentrations of external ions (S_{ex}) that are defined by the constant, q_s:

$$S_{ns} = q_s S_{ex}. \quad (2)$$

Values of q_s of lymphocyte ions are 0.6 for potassium, 0.2 for sodium, 0.4 for chloride and 0.6 for magnesium [24, 29, 38].

The second kind of observation that forms the basis for the adsorption model of the lymphocyte in Figure 2 includes the bi- or multi-exponential nature of isotopic ion self-exchange, in which fast fractions exchange in series with slower ones [24, 30 ,31] (Figure 1). As mentioned in the last part of the Introduction, the fast fractions represent exchange of ions across the surface membrane. The amounts of ions exchanging in the fast fractions are the same as the amounts present in the non-saturable fractions of static ion contents [24, 30-35]. The amounts of ions exchanging in the slow fractions are the same as the amounts present in the saturable fractions. Thus, when sodium replaces potassium, which it does in a mole-for-mole fashion at low temperature, in low external potassium, or with metabolic perturbation, the accumulated sodium exchanges within the slowest fraction [31, 34, 35, 39].

The adsorption model of the lymphocyte in Figure 2, derived from the abovementioned studies, is



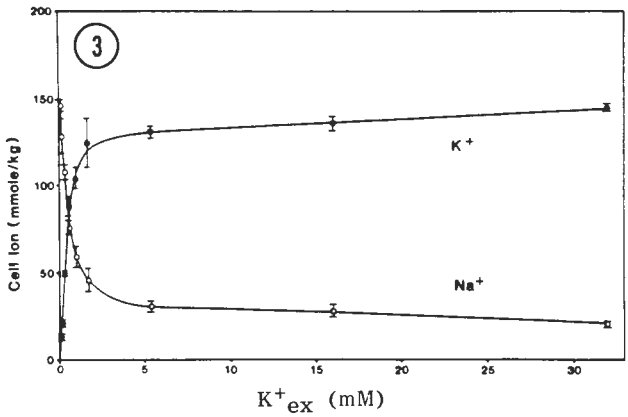


Figure 2 (at left). Schematic model of the lymphocyte, based on static ion content and isotopic ion exchange data. The zig-zag line indicates macromolecules that adsorb ions. Free ions are dissolved in ordered cell water, and exchange with external ions through the cell membrane. Thick arrows indicate fast fractions of exchange (F in Figure 1), and thin arrows indicate slower exchange between adsorbed and free ions (S in Figure 1).

Figure 3 (at right). Equilibrium, static potassium and sodium contents of lymphocytes at 37°C., where external K + Na was constant at 150 mM. Redrawn from reference 29.

not original but conforms very closely to G.N. Ling's theory of the cell, the association-induction hypothesis [15, 19]. It has two fundamental concepts:

(1) Most of cell water exists in a physical state sufficiently ordered that it reduces the partition function of solutes dissolved within it. This mechanism tends to exclude solutes so that their concentrations in cell water are less than in the external medium. This is the basic mechanism for solute exclusion from the cell, and affects different solutes to different degrees depending on their size, molecular configuration, and presence or absence of hydrogen-binding groups. It is responsible for the non-saturable fractions of cell ions having different q values. A corollary of this concept is that the rate of exchange of these freely dissolved solutes is determined by the permeability of the surface membrane, and that this is represented by the fastest fractions of isotopic ionic self-exchange. In the lymphocyte, these fractions of potassium, sodium, and chloride all have half-times of about 2 minutes, which, given the known size and surface area of these cells indicates permeability coefficients on the order of 10^{-6} cm/sec. These coefficients are typical of those reported in other cells and in phospholipid-protein vesicles, some of which are listed in Table 1.

(2) Ions closely asociate with fixed-charge groups on cell proteins. This mechanism may result in ion accumulation to concentrations greater than in the external medium. Selective ion accumulation depends on the short-range electrostatic properties of the fixed-charge groups and the nature and chemical activities of competing counterions. This mechanism accounts for the high level of potassium in most cells, and its mole-for-mole replacement by sodium under a variety of conditions. A corollary of this concept is that the rate of exchange of these ions is determined by their rates of adsorption onto and desorption from fixed-charge groups. If this exchange is slower than the rate of movement of the ions across the surface membrane, then it will appear as a slower fraction in isotopic ion self-exchange experiments. Experimentally, this is what we observe

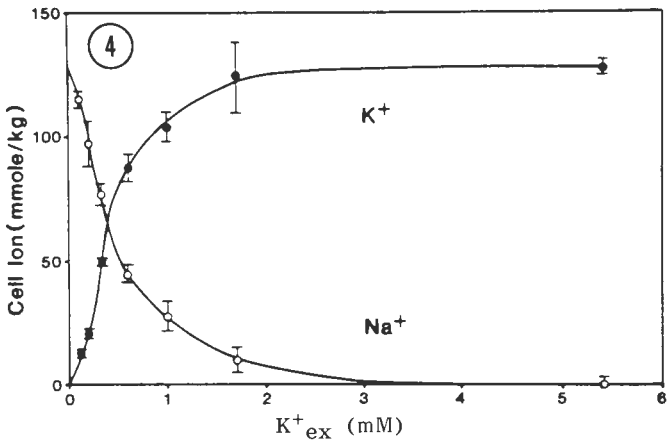
Table 1. Membrane Permeability Coefficients (cm/sec). Those in the human lymphocyte are derived from fast fractions of isotopic ion self-exchange as described in the text. Those of other cells are from Table 5-9 of Jain [8].

	K^+	Na^+	Cl^-
Human Lymphocyte	10^{-6}	10^{-6}	10^{-6}
Frog Skeletal Muscle	10^{-7}	10^{-7}	10^{-7}
Squid Giant Axon	10^{-7}	10^{-8}	10^{-8}
Human Erythrocyte	10^{-10}	10^{-10}	10^{-4}
Phospholipid Vesicle		10^{-7}	

in the human lymphocyte (Figure 1). In addition, we observed that this fraction of exchange is the one affected in amount and rate by temperature, chemical potential, and metabolic perturbation. We assume therefore than these factors affect primarily the interaction between adsorbed ions and fixed charges.

Concepts of the physical state of cell water in this model, and its relation to cell volume control, were the subject of a Symposium in a prior Scanning Microscopy meeting [25]. Physical studies of the state of potassium in cells are described in other papers in this journal (to be separately published later as a special compilation) derived from the Symposium of which this article is a part, and include electron microscopic, autoradiographic and laser-mass microprobe analyses of striated muscle [2]; near-edge x-ray absorption studies in red cells [6]; and diffusion profile analyses of open-ended muscle cells [18].

A detailed molecular theory of ion-fixed charge association was presented by Ling [15]. His theory takes into account all electrostatic interactions and uses a Born charging method to calculate the adsorption energies of the monovalent cation series as functions of electron density and polarizability of the



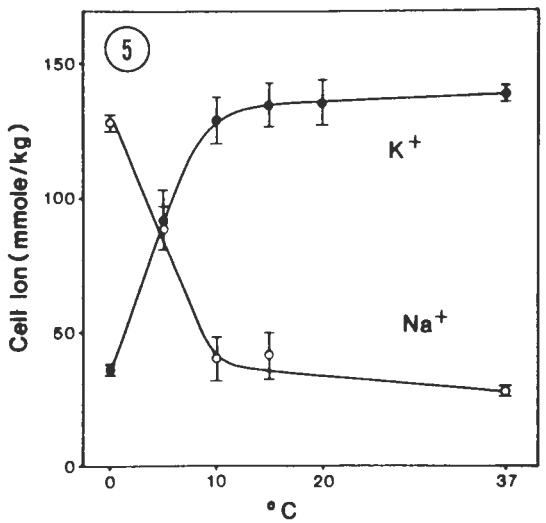


Figure 4 (at left). Equilibrium isotherm of the saturable fractions of ions from Figure 3.

Figure 5 (at right). Temperature dependence of equilibrium ion contents of lymphocytes. External K 5.5 mM, Na 144.5 mM. Redrawn from reference 32.

fixed, oxyacid adsorption sites. The adsorption energies are weighted according to the Boltzmann distribution of four configurations of the linear complex of the oxyacid and the counterion with the interposition of 0 to 3 water molecules between them. This theory provides the basis for the understanding of the reversal of selectivity for counter-cations, such as when sodium replaces potassium [15]. Details of the application of this concept are reviewed in Ling's recent book [19].

The remainder of this article reviews evidence for a critical, cooperative interaction between ion-adsorbing sites in lymphocytes, and its relation to the kinetics of ion exchange. A detailed account of this topic was published recently [26].

The Cooperative Nature of Potassium Adsorption

The cooperative adsorption model describes the free energy of ion adsorption over the entire ensemble of ion-adsorbing proteins as a function of the chemical potentials of the ions in the external medium. A one-dimensional Ising model has been found most useful to describe the ensemble of interacting adsorption sites and to predict its behavior [12, 16, 17]. For our purposes, an adsorption site exists in either the potassium state or the sodium state, and its occupancy at any one moment is determined by the intrinsic property of the site, the differences between the chemical potentials of potassium and sodium, and the degree of interaction with near-neighboring sites. The status of each ion in the ion-adsorbing ensemble is described by a statistical mechanical partition function which contains the energy required to desorb the ion, the chemical potential of the ion, and a rotational component. The grand partition function enumerates the states of the total of S_t sites. The ratio of partition functions of potassium and sodium is designated by the parameter ξ. The energy of interaction between near-neighboring sites is designated $-\gamma/2$. The concentration of adsorbed potassium is:

$$K^+_{ad} = \frac{f_T}{2}\left[1 + \frac{(\xi - 1)}{\sqrt{(\xi - 1)^2 + 4\xi e^{\gamma/RT}}}\right] \quad (3)$$

A similar expression describes the adsorption of sodium. Taking the ratios of these expressions permits one to express the competing adsorptions of potassium and sodium by a variant of the familiar Hill equation:

$$\ln (K^+_{ad}/Na^+_{ad}) = n \ln (K^+_{ex}/Na^+_{ex}) + n \ln \tilde{K}_{K,Na}, \quad (4)$$

where $\tilde{K}_{K,Na}$ is the free energy of association in the exchange of potassium for sodium, and n expresses the energy of near-neighbor interaction: $(n = e^{-\gamma/2RT})$.

If there is no interaction between adsorbing sites, n = 1 and the saturable fractions of potassium and sodium follow Langmuir isotherms. If sites interact such that the adsorption of one species of ion reduces the affinity of a neighboring site for that species, n < 1 and the adsorption isotherms are heterocooperative. If sites interact such that adsorption of one species of ion increases the affinity of neighboring sites for that species, n > 1 and the adsorption isotherms are cooperative. The steep, sigmoidal nature of the saturable fractions of potassium and sodium in lymphocytes (Figure 4) is compatible with a cooperative interaction between adsorption sites. For these data, n = 3.0, and the transition point, at which half of the sites adsorb potassium and half sodium, occurs at 0.4 mM K^+_{ex} and 149.6 mM Na^+_{ex}. Hence, the free energy of adsorption is 3.5 kcal/mole and the free energy of nearest-neighbor interaction is 0.66 kcal/mole. Similar observations have been made in a variety of muscle cells [9-11, 20].

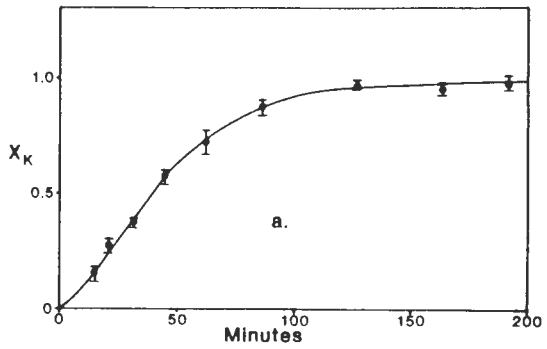


Figure 6. Time course of uptake of potassium by lymphocytes. Cells had been loaded with Na and depleted of K, then transferred to medium containing 5.4 mM K, 144.6 mM Na at 37°C, labelled with ^{42}K.

Critical Temperature Transition of Potassium Adsorption

One aspect of the behavior of the cooperatively-interacting ion-adsorbing ensemble is its critical temperature dependence. That is to say, the transition between potassium- and sodium-adsorption occurs in a relatively abrupt fashion. This is shown experimentally in Figure 5 where it is noted that the cell contents of these ions remain unchanged to 10°C, and then abruptly change between 10 and 0°C. Similar behavior has been reported in smooth muscle, Ehrlich cells, and a bacterium [1, 40, 41]. This phenomenon is subject to description by the thermodynamic theory of phase transitions of Landau [4], which predicts that at a temperature below 10°C, the adsorption isotherm of potassium in lymphocytes will simply be shifted rightward relative to that at 37°C in Figure 4. This has been experimentally confirmed [32].

Other Perturbations of the Ion-Adsorbing Ensemble

One of the agents commonly used to perturb cell potassium and sodium is the cardiac glycoside, ouabain. The reason for this is that ouabain is known to inhibit the Na,K-ATPase, which in classical membrane theory is assumed to be the sodium pump in most cells. Indeed, ouabain does cause cells, including lymphocytes [27] to replace potassium by sodium. In context of the adsorption model (Figure 2), ouabain affects the adsorbed fractions of ions, and we have shown that it simply shifts the ion adsorption isotherms (Figure 4) rightward. Hence, its behavior is analogous to that of an allosteric effector. Detailed studies of this phenomenon, and its relation to the association-induction hypothesis, have been reported by Ling and Bohr in frog muscle [21].

Temperature and ouabain appear, within experimental error, to affect only the adsorbed fractions of potassium and sodium, and not the non-saturable fraction dissolved in cell water or the permeability of the surface membrane [35]. In contrast, metabolic inhibition with ATP depletion causes both replacement of adsorbed potassium by sodium and an increase in the amount of sodium dissolved in cell water [23]; the latter is assumed to result from a loss of water-ordering in the cell, and to be associated with the ease with which these cells can be made to swell [23].

Kinetics of the Approach to Equilibrium in the Cooperative Ion-Adsorbing Ensemble

To describe the time-dependent behavior of the cooperatively - interacting ion adsorption sites, one assumes that the ensemble will move from one temporary equilibrium state to another, permitting use of Glauber's time-dependent Ising model to describe the kinetics of the approach to equilibrium in the exchange of potassium for sodium [13]. The probability that a variable of state changes from s_i to $-s_i$, or from $-s_i$ to s_i, in the time Δt is related to the probabilities of the equilibrium states, $p(s_i)$ and $p(-s_i)$. The time derivative of the probability of N sites is given by Glauber's equation:

$$\frac{d}{dt}\, p(s_1,\ldots,s_N,t) =$$

$$-\left[\sum_{i=1}^{N} w_i(s_i)\right] p(s_1,\ldots,s_N,t)$$

$$+\left[\sum_{i=1}^{N} w_i(-s_i)\right] p(s_1,\ldots,-s_i,\ldots s_N,t) \qquad (5)$$

where $p(s_1,\ldots,s_N,t)$ includes all variables that did not change in the time Δt, and $p(s_1,\ldots,-s_i,\ldots,s_N,t)$ includes all variables that changed from $-s_i$ to s_i in the interval Δt, for Δt approaching the limit of zero. $w_i(s_i)$ and $w_i(-s_i)$ are the probabilities per unit time that a variable of state, s, changes from s_i to $-s_i$, or from $-s_i$ to s_i. The functions $d/dt\, p(s_1,\ldots,s_N,t)$ are solved by knowing the functions of $w_i(s_i)$ and the initial values of the probabilities $p(s_1,\ldots,s_N,t)$. Here, $w(s_i)$ is the probability that a site changes from the K^+ state to the Na^+ state, and $w(-s_i)$ is the probability that a site changes from the Na^+ state to the K^+ state. These are related to the equilibrium parameters, ξ and n (equations 1 and 2) in the following manner:

$$w_i(s_i) = A\, n^{-[s_i(s_{i-1} + s_{i+1})/2]}\, \xi^{-s_i/2} \qquad (6a)$$

$$w_i(-s_i) = A\, n^{[s_i(s_{i-1} + s_{i+1})/2]}\, \xi^{s_i/2} \qquad (6b)$$

where A is a proportionality factor. The total time rate of the probability of change of the variable of state, s_i, is characterized by the parameter, α, which is the ratio between a time constant and real time, $\alpha = \tau/t$. α is related to the equilibrium parameters, ξ and n, as follows:

$$\alpha = A\,(n^{\frac{1}{2}} + n^{-\frac{1}{2}})\,(\xi^{-\frac{1}{2}} + \xi^{\frac{1}{2}}) \qquad (7)$$

These relations are then combined with Glauber's equation, equation 5, to give the time derivative of the probability of the state of N sites. The resulting differential equations are readily solved to describe the approach to equilibrium from an initial state.

To test this approach in lymphocytes, the sodium-adsorbing state was chosen as the initial one. Cells were depleted of potassium and loaded with sodium, and then placed in media containing 5.4 mM K^+, 145 mM Na^+ at 37°C, and the re-uptake of potassium followed. Under these conditions the equilibrium parameters, ξ and n, are known from the experiment in Figure 4. The kinetic data are shown in

Figure 6. The curve is theoretical, fitted only by normalizing to the time of half-saturation [28].

More recently, Huang obtained a closed solution of Glauber's kinetic Ising model with variable chemical potential [5], and we have found that our data fits his solution as well [7].

The results of this study are significant, first because they show a rigorous relationship between equilibrium and kinetic parameters of cell ion exchange, and second because they reinforce the impression that a cooperative interaction between ion-adsorbing sites modulates their affinities for potassium and sodium.

Ionic Self-Exchange with Cooperatively-Interacting Sites

In the kinetics of the approach to equilibrium described in the preceding section, net changes in cell ion concentrations are occurring. Ionic self-exchange refers to exchange of one species of ion for itself, and is determined experimentally by isotopic influx or efflux techniques under conditions in which the ion contents remain static. Examples of isotopic efflux studies were shown in Figure 1. The slowest exponential fractions (S) are the ones of interest; when sodium replaces potassium, it exchanges within this fraction. The effects of temperature and chemical potential on ionic self-exchanges will give some insight into one aspect of the behavior of the ion-adsorbing ensemble.

The effect of temperature on ionic self-exchanges is shown in Figure 7. The static ion contents under the same experimental condition are shown in Figure 4. Comparison of these figures shows that self-exchange of potassium has a steep temperature-dependence when the ion-adsorbing sites are loaded with potassium, and self-exchange of sodium has a steep temperature-dependence only when sodium has replaced potassium on these sites. One may predict, then, that if the ensemble of adsorption sites is induced to replace potassium by sodium over the entire temperature range of 0 to 37°C, then the self-exchange of sodium will acquire a steep temperature-dependence over this entire range. The experimental results in Figure 8, in which either ouabain or zero external potassium were used, confirm this expectation.

To study the effect of chemical potential on ionic self-exchange on and off the cooperatively-interacting ensemble of sites, we took advantage of the fact that at 37°C, sodium replaces potassium only when external potassium is reduced below 2 mM (Figure 4). When equilibrium is reached between 0 and 2 mM external potassium, some of the sites adsorb potassium and some sodium. Self-exchange of potassium on these sites can be observed at all external potassium levels above 0, but self-exchange of sodium on these sites can be observed only below 2 mM external potassium. The experiments in Figure 9 show that both potassium and sodium have rates of exchange that rise to peaks at 0.5 mM external potassium or below. This is very close to the point at which half of the sites adsorb potassium and half sodium (Figure 4), which occurs at 0.4 mM external potassium.

Why do the rates of self-exchange of potassium and sodium become high near the transition point? Elsewhere [26], I have speculated that this may be a manifestation of microscopic fluctuations within the cooperative ensemble of ion adsorption sites. Indeed, it is a well known property of critical phenomena that near a transition point the ensemble may be very susceptible to slight changes in external parameters, at which point foci of correlated elements appear within the ensemble. An easily visualized example is critical opalescence of water, which is due to focal inhomogeneities in density with correlation lengths on the order of the wavelength of light [44].

In the cooperative ion-adsorbing ensemble, fluctuations may be present in which changes of adsorption state occur within foci of adsorption sites. Near the transition point of potassium-sodium adsorption at 0.4 mM external potassium, a higher rate of fluctuations may occur. There are as many sites changing from a potassium to a sodium state as there are sites changing from a sodium to a potassium state, so that from a macroscopic standpoint the ion contents are static and the system appears at equilibrium. However, the intrinsic rate of net exchange of potassium for sodium, or of sodium for potassium, within a focus of interacting sites (half-time 40 minutes, Figure 6) is higher than the rate of self-exchange of potassium for potassium or of sodium for sodium on the sites not undergoing net ion exchanges (half-times 210 and 120 minutes, respectively, Figure 1). Since the experimentally observed isotopic exchange is a weighted average of ion exchange with all microscopic elements within the ensemble, a high rate of fluctuations between ion-adsorbing states near the transition point will result in an increase in the rate of ionic exchange observed macroscopically.

This concept, if correct, would help explain several otherwise unexplained observations. These include the high rate of exchange of potassium at low external potassium described in Ehrlich cells [3] and canine carotid artery muscle [11]. It is conceivable that certain experimental manipulations may affect ion exchanges by inducing an increased rate of fluctuations without affecting static ion contents; this is the case for the effect of mitogens on the rate of potassium exchange in lymphocytes [42] and for the effect of valinomycin on the rate of sodium exchange in lymphocytes [37]. Hence, it is conceivable that, viewed from this particular theoretical perspective, the isotopic ion exchange experiment may be a means to examine the microscopic states of the ion-adsorbing ensemble.

Summary: Properties of the Cooperative Ion Adsorption Ensemble

A wide variety of experimental observations in lymphocytes are readily interpreted in the context of the adsorption model of the cell (Figure 2). A steep, sigmoidal equilibrium isotherm of potassium and sodium, described by a one-dimensional Ising model, provides the primary evidence for a critical, cooperative interaction between ion-adsorbing sites. Other manifestations of the static ion contents that are in accord with this include the critical temperature transition of sodium-potassium exchange and the "allosteric" effects of low temperature and of ouabain, which shift the equilibrium cooperative adsorption isotherms toward a higher chemical potential of potassium. The kinetics of net exchanges of potassium for sodium in the approach to equilibrium are

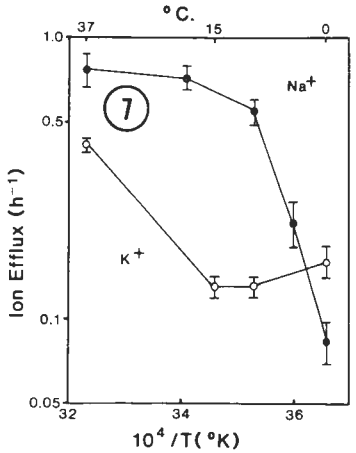
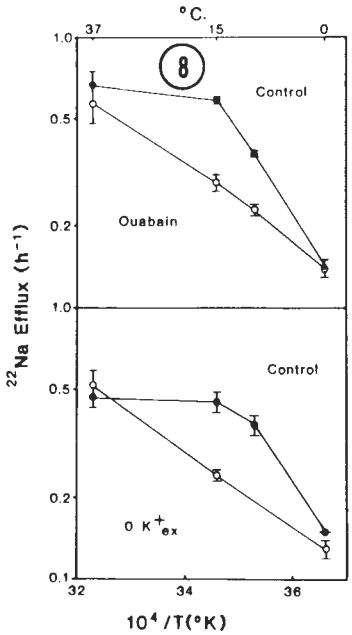


Figure 7 (above). Temperature dependence of the rates of the slow components of ion self-exchange in lymphocytes, determined by isotopic efflux techniques as in Figure 1. Redrawn from references 35 and 39.



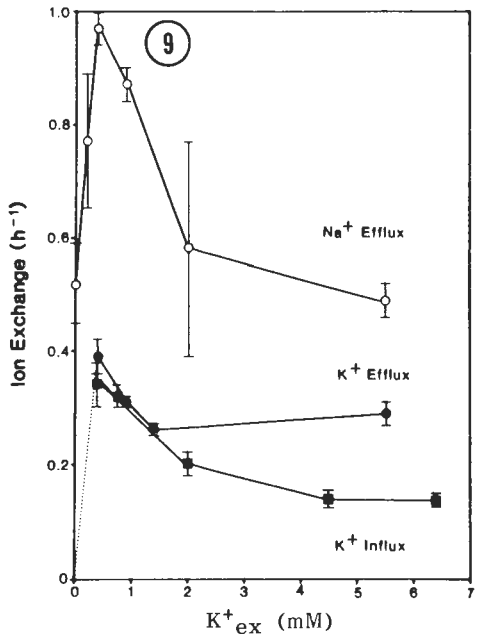


Figure 8 (center). The effects of low external potassium and of ouabain on the temperature dependence of self-exchange of sodium in lymphocytes. Redrawn from references 35 and 39.

Figure 9 (at right). Rates of self-exchanges of ions on the cooperatively interacting adsorption sites as a function of chemical potential ratios. External K + Na was 150 mM. Redrawn from references 30 and 39.

described by a kinetic Ising model with only a single curve-fitting variable. The self-exchange of sodium on the cooperatively interacting sites attains properties like those of potassium, when potassium is occupying them. These include a steep temperature-dependence and a high rate near the transition point. Finally, self-exchange of sodium has different rates that vary with the means by which sodium is induced to replace potassium. It is postulated that with the theoretical background provided by this model, the simple ionic self-exchange experiment may provide a means to explore the microscopic state of the ion-adsorbing ensemble with minimal perturbation of the cell.

Acknowledgement

Studies of lymphocytes described in this review were supported by Office of Naval Research Biophysics contract N00014-76C-1166.

References

1. Damadian R, Goldsmith M, Zaner KS. (1971) Biological ion exchanger resins. Biophys. J. 11, 761-772.

2. Edelmann L. (1984) Subcellular distribution of potassium in striated muscles. Scanning Electron Microsc. 1984; II, 875-888.

3. Hempling HG. (1962) Potassium transport in the Ehrlich mouse ascites tumor cell: evidence for autoinhibition by external potassium. J. Cell. Comp. Physiol. 60, 181-198.

4. Huang HW. (1977) Thermodynamics of allosteric transitions. J. Theor. Biol. 67, 557-565.

5. Huang HW. (1979) Hydrodynamic solution of a time-dependent Ising model. J. Chem. Phys. 70, 2390-2391.

6. Huang HW, Hunter SH, Warburton WK, Moss SC. (1979) X-ray absorption edge fine structure of potassium ions in various environments: application to frog blood cells. Science 204, 191-193.

7. Huang HW, Negendank W. (1980) Experimental test of the time-dependent Ising model. J. Chem. Phys. 73, 4136-4137.

8. Jain MK. (1972) The Bimolecular Lipid Membrane. Van Nostrand, New York.

9. Jones AW (1970) Effects of progesterone treatment on potassium accumulation and permeation in rabbit myometrium. Physiol. Chem. Phys. 2, 151-167.

10. Jones AW (1973) Control of cooperative K accumulation in smooth muscle by divalent ions. Ann. New York Acad. sci. 204, 379-389.

11. Jones AW, Karreman G. (1969) Ion exchange properties of the canine carotid artery. Biophys. J. 9, 884-909.

12. Karreman G. (1965) Cooperative specific adsorption of ions at charged sites in an electric field. Bull. Math. Biophys. 27, 91-104.

13. Karreman G. (1971) Stochastic treatment of cooperative specific adsorption. Bull. Math. Biophys. 33, 483-495.

14. Leaf A. (1956) On the mechanism of fluid exchange of tissues in vitro. Biochem. J. 62, 241-248.

15. Ling GN. (1962) A Physical Theory Of The Living State. Blaisdell, Waltham, MA.

16. Ling GN. (1964) The role of inductive effect in cooperative phenomena of proteins. Biopolymers Symp. 1, 91-116.

17. Ling GN. (1966) All-or-none adsorption by living cells and model systems. Fed. proc. 25, 958-970.

18. Ling GN. (1978) Maintenance of low sodium

and high potassium levels in resting muscle cells. J. Physiol. 280, 105-123.

19. Ling GN. (1984) In Search Of the Physical Basis Of Life. Plenum Press, New York.

20. Ling GN, Bohr G. (1970) Studies on ion distribution in living cells. II. Cooperative interaction between intracellular potassium and sodium ions. Biophys. J. 10, 519-538.

21. Ling GN, Bohr G. (1971) Studies on ion distribution in living cells. III. Co-operative control of electrolyte accumulation by ouabain in the frog muscle. Physiol. Chem. Phys. 3, 431-447.

22. Negendank W. (1982) Studies of ions and water in human lymphocytes. Biochim. Biophys. Acta 694, 123-161.

23. Negendank W. (1984) Ionic distributions and volume maintenance in human lymphocytes. Physiol. Chem. Phys. 16, 3-20.

24. Negendank W. (1984) The permeability of human lymphocytes to chloride. Biochem. Biophys. Res. Commun. 122, 522-528.

25. Negendank W and Edelmann L. (1988) The State Of Water In the Cell. Scanning Microscopy International, AMF O'Hare (Chicago), IL.

26. Negendank W. (1988) A cooperative transition theory applied to the kinetics of ionic exchanges in cells. Cell Biophysics 13, 93-117.

27. Negendank W, Collier C. (1976) Ion contents of human lymphocytes: The effects of concanavalin A and ouabain. Exp. Cell Res. 101, 31-40.

28. Negendank W, Karreman G. (1979) Rate of potassium-sodium exchange by human lymphocytes: prediction of the cooperative adsorption model. J. Cell. Physiol. 98, 107-112.

29. Negendank W, Shaller C. (1979) Potassium-sodium distribution in human lymphocytes: Description by the association-induction hypothesis. J. Cell. Physiol. 98, 95-106.

30. Negendank W, Shaller C. (1979) Fast and slow fractions of K^+ flux in human lymphocytes. J. Cell. Physiol. 98, 539-552.

31. Negendank W, Shaller C. (1980) Multiple fractions of sodium exchange in human lymphocytes. J. Cell. Physiol. 104, 443-459.

32. Negendank W, Shaller C. (1980) A critical temperature transition of K^+-Na^+ exchange in human lymphocytes. J. Cell. Physiol. 103, 87-95.

33. Negendank W, Shaller C. (1981) Simultaneous net accumulation of both K and Na by human lymphocytes at 0° C. Biochim. Biophys. Acta 640, 368-373.

34. Negendank W, Shaller C. (1982) The effect of metabolic inhibition on ion contents and sodium exchange in human lymphocytes. J. Cell. Physiol. 110, 291-299.

35. Negendank W, Shaller C. (1982) The effects of temperature and ouabain on steady-state Na and K exchanges in human lymphocytes. J. Cell. Physiol. 113, 440-454.

36. Negendank W, Shaller C. (1982) Temperature-dependence of ATP level, organic phosphate production and Na, K-ATPase in human lymphocytes. Physiol. Chem. Phys. 14, 513-518.

37. Negendank W, Shaller C. (1982) Effects of valinomycin on lymphocytes independent of potassium permeability. Biochim. Biophys. Acta 688, 316-322.

38. Negendank W, Shaller C. (1983) Mg^{++} distribution in human lymphocytes. Biophys. J. 41, 190a.

39. Negendank W, Shaller C. (1984) Self-exchange of sodium in human lymphocytes. Biophys. J. 46, 331-342.

40. Reisin IL, Gulati J. (1972) Cooperative critical thermal transition of potassium accumulation in smooth muscle. Science 176, 1137-1139.

41. Reisin IL, Gulati J, Ling GN. (1971) Critical cooperative transition of electrolyte accumulation with temperature. Fed. Proc. 30, 331.

42. Segel GB, Lichtman MA. (1976) Potassium transport in human blood lymphocytes treated with phytohemagglutinin. J. Clin. Invest. 58, 1358-1369.

43. Skou JC. (1957) The influence of some cations on adenosine triphosphatase from peripheral nerves. Biochim. Biophys. Acta 23, 394-401.

44. Stanley HE. (1971) Introduction To Phase Transitions And Critical Phenomena. Oxford University Press, New York.

45. Wilson TH. (1954) Ionic permeability and osmotic swelling of cells. Science 120, 104-105.

Discussion With Reviewers

H.E. Rorschach: Is the slow fraction of the efflux rates due to a (variable) absorption time of an ion on a site followed by normal diffusion as for the fast fraction? Or is the slow efflux rate made up of a number of repeated absorption and desorption processes as the ion moves through the cell from one site to another? The latter would seem to be more consistent with the results of Figure 9.

Author: I believe that the latter mechanism, absorption and desorption as the ion moves through the cell from one site to another, is the more likely mechanism underlying the slow fractions. However, a detailed physical model of the process has not yet been developed.

T. von Zglinicki: The data you present in Figures 3 and 4 should be comparable to ours which independently demonstrate saturated ion binding in isolated lymphocyte nuclei (Scanning Microscopy, accepted for publication). In order to enable the reader to do that please state: How large is the water content in these cells? How were water and ion content measured?

Author: The water content of "control" cells in Hank's medium at 37°C was 77.8% wet weight. It was not significantly different under the other experimental conditions mentioned. Water was measured by drying cell pellets at 100°C to constant weight. Ions were measured by atomic absorption spectroscopy of 0.1 N HCl extracts.

T. von Zglinicki: Is the slope of the regression line for the nonsaturable fraction (the q value) of K significantly different from one? In other words, is the water within lymphocytes so highly ordered that significant exclusion of K^+ takes place?

Author: The slope of the nonsaturable fraction of potassium was shown to be significantly different from zero in studies up to 64 mM external K^+ (29). The non-saturable fraction was shown to correspond to the rapidly-exchanging fraction ($r = 0.82$, p 0.02), and to be the same as determined by the quite different techniques of isotopic influx and efflux (30). In addition, agreement between observed and expected sizes of fast and slow fractions was found in cells incubated at 0°C [32]. Thus, we are fully convinced of the reality of the non-saturable fraction of

potassium and, in context of the model in Figure 2, the ability of cell water to exclude it to a concentration 0.6 that of the external medium.

T. von Zglinicki: What is the intermediate exchange fraction of Na^+ (Figure 1) ?
Author: The intermediate fraction of exchange of Na^+ has been characterized experimentally in some detail [39]. It contains about 25% of the normal amount of cell sodium, is saturated (i.e., does not increase in absolute terms when the cells gain Na^+), has little or no temperature-dependence of its rate of exchange, and is independent of the external K^+ concentration. For all these reasons, it is clearly separate from either the fast or the slow fractions of Na^+. Its origin, however, is not known. It is not chelated (i.e., chemically bound) since its halftime of exchange is 10 - 12 minutes. It could represent a saturated set of binding sites anywhere in the cell, or it could be confined to smaller membranous organelles (eg., mitochondria or vesicles). We have evidence that approximately this amount of Na^+ is retained by detergent (Brij)-treated lymphocytes (Biophys. J. 47:397a, 1985), and it may in fact be contained within the nucleus, since Itoh and Schwartz (Am.J.Physiol. 188:490, 1957) found considerable Na^+ retained in the TCA-precipitate of calf thymus lymphocyte nuclei.

H.G. Hempling: Legend to figure 2: Is Dr. Negendank accepting the hypothesis that ions may dissolve in ordered water?
Author: Yes. This was stated explicitly in my paper in the section entitled "An Adsorption Model of Cell Potassium and Sodium", as well as in the legend of Figure 2.

H.G. Hempling: Is Dr. Negendank also accepting a definitive structure called a membrane that delimits the lymphocyte from its environment? Does he think, perhaps, that this limiting structure may separate charge and give rise to a potential difference across the membrane? If so, is he prepared to explain the electrophysiological data which Deutsch and her colleagues have published and correlated with concomitant studies on volume regulation?
Author: Certainly, I believe there exists a surface membrane around the lymphocyte; in fact, I have interpreted the fast fractions of ionic self-exchange as being membrane-limited and have derived the corresponding membrane permeability coefficients mentioned explicitly in the paper and included in Table 1. I believe that the electrical potential of cells is a surface phase-boundary potential and that its mechanism is related to selective ionic adsorption [see Chapter 14 in reference 19]. A theory of volume regulation based on the concepts presented here, has been published [22, 23], and I see no contradiction between the data which led to Deutsch and Lee's model (Renal Physiol. Biochem. 5:260-276, 1988) and this theory.

R.L. Post: I think that this treatment of the pump-leak hypothesis (in the Introduction) is sketchy and partly incorrect. For instance, take item 1, inhibition of the pump only permits cell swelling. Swelling is also a function of leak pathways. Thus the action of the pump is only one factor influencing cell volume. The author writes that when swelling failed to appear under conditions that inhibited the pump, he could not account for this result. A simple explanation for the result is that the cells lost potassium through leak pathways in an amount approximately equal to the gain in sodium. Thus there was no change in ionic or osmotic content of the cells and no change in volume would be expected by the osmotic theory. This point and related topics are considered by A.D.C. Macknight and A. Leaf in an article entitled Regulation of Cellular Volume, Chapter 17, pages 315-334 in a book, Physiology of Membrane Disorders, T.E. Andreoli, J.F. Hoffman, and D.D. Fanestil (eds.), published by Plenum in 1978.
Author: The full statement I made in item 1 was that inhibition of the sodium pump permits cell swelling due to the forces involved in the Donnan equilibrium. Loss of potassium through leak pathways in an amount approximately equal to the gain in sodium is not a sufficient condition to counterbalance the forces at work in a Gibbs-Donnan system. This idea, crucial to concepts of volume regulation, was clearly stated by the very authors that Dr. Post has cited. On page 319 of their chapter, Macknight and Leaf state "The volume that cells maintain is thus seen to represent a steady state, maintained by metabolism, in which the total number of osmotically active cellular constituents is kept constant. This constancy is critically dependent on sufficient exclusion of sodium from cells to counterbalance the cellular colloid osmotic pressure." Incidentally, Macknight and Leaf's chapter was expanded into a detailed review published in Physiological Reviews in 1977 (volume 57, pages 510-572). This supurb review is recommended for all students of this subject. It must be emphasized that in my experiments in the lymphocyte, the cell sodium and other ion levels were permitted to reach new, stable concentrations, and the cellular sodium was equal to or greater than that in the external medium. By this very fundamental criterion, outward pumping of sodium by any mechanism is ineffective as a means to regulate volume. For details, the reader is referred to the literature cited in my paper.

R.L. Post: The author might note that the adsorption model featured later in the review provides no mechanism to account for changes in cell volume reported in the literature, particularly those appearing in response to changes in extracellular osmotic pressure.
Author: A theory of volume regulation based on the concepts presented in my paper has been published (references 22 and 23).

R.L. Post: Next take item 2, the gain of cell sodium caused by inhibition of the sodium pump. The author could not account for an increase in sodium efflux that appeared as the intracellular sodium concentration increased in inhibited cells. He thought that inhibition of the pump should stop all sodium efflux. The pump generates only one component of efflux. Other components pass through leak and exchange pathways. The component of efflux that goes through leak pathways is expected to increase as the sodium content of the cell increases. In the simplest sort of leak model this would be in accordance with the Ussing equation.
Author: It is not correct to say that I "thought that inhibition of the pump should stop all sodium

efflux". Indeed, in the primary experimental papers cited in the Introduction I analyzed the results according to a variety of postulated exchange pathways, and the reader is referred to these papers for details. In the case of metabolic inhibition with iodoacetate and nitrogen (reference 34) I studied sodium exchanges both during the gain of sodium and after a new stable level of sodium had been reached. During the gain of sodium, I measured the net sodium gain by atomic absorption spectrophotometry, the isotopic sodium influx, and the isotopic sodium efflux. The results showed no change in the rate of isotopic sodium efflux in the face of a matched increase in net and isotopic sodium influx. Thus, a small increase in the rate of sodium efflux (expressed in mmoles/kg/hr) occurred, and this might be expected as mentioned by Dr. Post. The point, however, is that the gain of sodium could in no way be attributed to an inhibition of outwardly directed sodium pumping. Moreover, once the cells gained sodium to high levels to dissipate the sodium gradient, the rate of sodium efflux (and hence also of influx) was markedly increased. One must postulate more than a simple leak model to explain why the influx increased after its major driving force, the electrochemical gradient of sodium, had dissipated. Finally, it is not clear what would be the significance of the results being in accordance with the Ussing equation. I assume Dr. Post is referring to Ussing's flux ratio equation (see Ussing,H.H., Interpretation of Tracer Fluxes, Chapter 3 in Membrane Transport in Biology, G. Giebisch, D.C. Tosteson and H.H. Ussing, (Eds.), Springer-Verlag, New York, 1978). Ussing's equation relates the natural logarithm of the ratio of the fluxes to the electrochemical potential differences on either side of a membrane. It is especially useful in studies of epithelial transport and often permits one to deduce whether or not active transport is occurring. However, in the case of the abovementioned studies with metabolically inhibited lymphocytes, during the gain of sodium I had complete data sets of net influx, isotopic influx and isotopic efflux, so use of the Ussing equation would not have added anything. Moreover, after the gradients had dissipated, the flux ratio would equal 1 (and its natural logarithm 0), so the Ussing equation could not have added any insight into mechanisms of exchange.

R.L. Post: The author refers to stimulation of a sodium pump by a high cytoplasmic sodium concentration when that pump is already inhibited by some other means. He is inconsistent in trying to stimulate an inhibited pump. The mention of internal negative charge, outward co-transport, counter-transport or exchange diffusion is also confusing. It is entirely in accord with membrane transport models that these mechanisms should not account for the increased sodium efflux. All that is needed is the simplest sort of leak pathway.

Author: It is not inconsistent to try to stimulate a pump inhibited by other means, since it is entirely reasonable to consider the competing influence of a variety of inhibiting and stimulating factors. The reason I mentioned this and the other potential mechanisms in my Introduction, and the reason I seriously considered and analyzed them in the primary experimental papers, is that they have all been postulated by other investigators to explain similar results in one or another kind of study in one or another kind of cell. In this regard, Dr. Post seems to be at odds more with his own colleagues in membrane physiology than with me!

R.L. Post: With respect to the third point, kinetic ion exchange studies, I would like to know the criteria by which the author came to those conclusions. How did the author show that the fast fractions were not in the extracellular medium, were not attached to the outer surface of the membrane, and did not arise from a population of cells? I would also like to know how he showed that the fast compartment was in series with the slow compartment. It seems to me that these points were not easy to establish.

Author: It is beyond the scope of my paper and of this discussion to deal with these points. Needless to say they are of crucial significance, for demonstration that the fast fraction of ionic exchange is limited by passage across the surface membrane, would eliminate the membrane as a controlling factor in the determination of cellular ion concentrations. This conclusion was reached as a result of detailed experiments described in the literature cited, in particular references 24 and 30-35.

R.L. Post: It seems to me that the author is going to have difficulty using his model to account for the effects of ouabain in his system. He is going to have to assume that ouabain passes through the cell membrane and binds to intracellular proteins with some stoichiometry relative to the binding of sodium and potassium. If the stoichiometry of ouabain to intracellular sodium and potassium in the lymphocytes is the same as for sodium and potassium ATPase, he could expect to find an intracellular ouabain concentration of about 30 to 50 mM.

H.G. Hempling: How does ouabain, whose receptors have been shown to be on the outside of the membrane, get to the ion-binding sites?

Author: I am sure the intracellular concentration of ouabain is not 30 - 50 mM, and that the effect of ouabain on lymphocyte sodium and potassium contents does not have the same stoichiometry as the ATPase. The fact that non-toxic concentrations of ouabain shift the sodium and potassium distribution isotherms rightward (reference 27) indicates that it has an allosteric effect, but it is not known if this is a direct effect or an indirect one, and whether it occurs via internal binding sites or is propagated cooperatively from membrane binding sites. In my opinion, it remains an open issue whether or not ouabain enters cells (Baker, P.R., and J.S. Willis, J. Physiol. 224: 441-462, 1972; Cook,J.S., Will, P.C., Proctor,W.R., and E.T. Brake, In: Biogenesis and Turnover of Membrane Macromolecules, J.S. Cook, editor, Raven Press, New York, pages 15-36). Indeed, in lymphocytes, the uptake of ouabain requires many hours to approach saturation (Segel, G.B., and M.A. Lichtman, J. Cell Physiol. 104: 21-26, 1980). It is certain, however, that in the lymphocyte ouabain does not simply inhibit the Na-,K-ATPase pump, as shown by studies of the temperature dependence of sodium and potassium exchanges (reference 35). In the presence of ouabain sodium exchange acquired a steep temperature dependence that it did not have in the absence of ouabain, while exactly the opposite occurred with potassium. These results are incompatible with coupled exchanges of sodium and potassium that would

have been expected were the ATPase responsible for their "pumping".

R.L. Post: I wonder if the association-induction hypothesis would be useful as a model for the mechanism by which sodium, potassium ATPase selectively binds sodium or potassium.
Author: Yes, I think it would. In addition, Ling has outlined a model of epithelial transport based on his hypothesis that incorporates the role of ion-activated ATPases (Chapter 17 of reference 19).

Reviewer V: This paper expounds a view of the organization of cellular ions which emphasizes the importance of intracellular structural organization (the Ling hypothesis) and rejects any significant role of the plasma membrane. While not denying the possible importance of intracellular macromolecules in affecting exchanges of ions between cells and interstitial fluid, it is impossible to ignore the fact that plasma membranes contain proteins which act as primary and secondary active transporters and as ion channels. Such proteins have now been isolated and reconstituted into lipid bilayers where their properties can be examined in the absence of any cellular macromolecules. While the author might argue that such proteins have not been prepared specifically from lymphocyte plasma membranes, it must be accepted that mechanisms as fundamental as those that determine cellular accumulation of potassium rather than of sodium and which determine cellular volume must be common to animal cells in general. For example, to base an argument for the Ling hypothesis on the absence of lymphocyte swelling after inhibition of the sodium pump ignores the fact that many other cell-types do swell after ouabain or metabolic inhibition. In addition, red cells from cats, for example, contain cellular proteins common to all mammalian red cells but are high in Na and low in K because they lack the enzyme (Na-K)-ATPase in their plasma membrane.

Author: The reviewer has raised questions of fundamental importance. I hope that the brevity of the Introduction to my paper does not lead the reader to think that I have taken these issues lightly. However, it is beyond the scope of the paper I wrote to discuss them and many of them have been addressed in considerable detail in the literature cited. The model of the cell shown schematically in Figure 2 leaves open the possibility that in other cells the permeability of the membrane may be low enough, and/or the rate of adsorption and desorption from internal sites fast enough, that observed ionic exchanges may be determined solely by the surface membrane. Indeed, this may be the case in human red cells for Na and K (but not for Cl) (see Table 1). The reconstituted membrane ATPase's have, to my knowledge, not been shown rigorously to pump ions according to the criterion that an electrochemical gradient of an ion be established or maintained in the face of leakage in the opposite direction. This issue, as well as the relation between Na/K exchanges in cells and properties of the Na,K-ATPase, were reviewed in some detail by Ling and myself (Perspectives in Biology and Medicine, 23:215-239, 1980). It is certainly true that cells other than lymphocytes swell when metabolically inhibited, and indeed lymphocytes can also swell under a variety of conditions. The observations I made in lymphocytes indicate that it is possible to create well-defined experimental conditions in which ion pumping can be shown rigorously not to be functioning in the manner needed to maintain volume, but in which the cells do not swell.